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Analysis of Catalases from Photosynthetic Bacterium *Rhodospirillum rubrum* S1

Hee-Kyoung Lim[1], Young-Mi Kim[1], Dong-Heon Lee[1], Hyung-Yeel Kahng[1,2] and Duck-Chul Oh[1,2]*

[1]*Department of Biology, College of Natural Sciences, and*
[2]*The Research Institute for Basic Sciences, Cheju National University, Jeju 690-756, Korea*

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Five different types of catalases from photosynthetic bacterium *Rhodospirillum rubrum* S1 grown aerobically in the dark were found in this study, and designated Cat1 (350 kDa), Cat2 (323 kDa), Cat3 (266 kDa), Cat4 (246 kDa), and Cat5 (238 kDa). Analysis of native PAGE revealed that Cat2, Cat3, and Cat4 were also produced in the cells anaerobically grown in the light. It is notable that only Cat2 was expressed much more strongly in response to the anaerobic condition. Enzyme activity staining demonstrated that Cat3 and Cat4 had bifunctional catalase-peroxidase activities, while Cat1, Cat2, and Cat5 were typical monofunctional catalases. S1 cells grown aerobically in the presence of malate as the sole source of carbon exhibited an apparent catalase Km value of 10 mM and a *Vmax* of about 705 U/mg protein at late stationary growth phase. The catalase activity of S1 cells grown in the anaerobic environment exhibited a much lower *Vmax* of about 109 U/mg protein at late logarithmic growth phase. The catalytic activity was stable in the broad range of temperatures (30°C-60°C), and pH (6.0-10.0). *R. rubrum* S1 was much more resistant to H_2O_2 in the stationary growth phase than in the exponential growth phase regardless of growth conditions. Cells of stationary growth phase treated with 15 mM H_2O_2 for 1 h showed 3-fold higher catalase activities than the untreated cells. In addition, L-glutamate induced an 80-fold increase in total catalase activity of *R. rubrum* S1 compared with malic acid. Through fraction analyses of S1 cells, Cat2, Cat3, Cat4 and Cat5 were found in both cytoplasm and periplasm, while Cat1 was localized only in the cytoplasm.

Key words: catalase, catalase-peroxidase, photosynthetic bacterium, *Rhodospirillum rubrum* S1

Rhodospirillum rubrum, a photosynthetic bacterium, has been used in various studies owing to its versatile metabolic abilities, especially energy-yielding metabolism, e.g., aerobic respiration, anaerbobic photosynthesis, and even fermentation (8, 38, 41, 45, 48, 53). It is well known that *R. rubrum* needs oxygen for its aerobic growth by respiration, but, in contrast, it needs strict anaerobic conditions for growth by photosynthesis or fermentation (50, 52). Oxygen is essential to all the aerobic microorganisms and even to facultative anaerobes for metabolism under aerobic conditions. However, it inevitably produces very reactive oxygen during aerobic growth, such as hydrogen peroxide, superoxide anion, or hydroxyl radicals that are toxic to cells (9, 11, 16, 25). Aerobes, facultative anaerobes, and even a few strict anaerobes produce several specific enzymes, such as catalase, peroxidase, superoxide dismutase, etc., to eliminate those toxic by-products. Among these enzymes, catalase has been extensively studied in various bacterial species (21, 23, 24, 26, 29-31, 42, 43, 47, 50, 56). Although many kinds of catalases have been purified and characterized from diverse bacterial species, we have little information and knowledge of catalases from photosynthetic bacteria (7, 10, 22, 24, 37, 50). Moreover, most catalases have been studied under aerobic conditions, while studies on catalases in response to the anaerobic conditions are very rare (14, 15, 42, 43, 55). In this respect, *R. rubrum* S1 capable of aerobic and anaerobic growth is considered a very suitable microorganism for comparative analysis of catalases in response to both aerobic and anaerobic conditions. In this study, we comparatively analyzed and characterized catalases produced by *R. rubrum* S1 grown aerobically in the dark and anaerobically in the light.

* To whom correspondence should be addressed.
(Tel) 82-64-754-3524; (Fax) 82-64-756-3541
(E-mail) duckoh@cheju.cheju.ac.kr

Materials and Methods

Bacterial strain and culture conditions

Rhodospirillum rubrum S1 (ATCC 11170) was used for this study. To analyze catalases produced under aerobic conditions, cells were pre-grown to the exponential phase

on 100 mL of a basal medium (3) supplemented with 0.3% (w/v) malic acid as the sole source of carbon and energy with shaking in the dark at 30°C, 150 rpm for 24 hours. Some of the pre-culture was transferred to another 100 mL of liquid media whose initial OD_{680} was adjusted to 0.05, and grown in the same condition, and analyzed for catalase activity by native PAGE. For anaerobic growth, media were prepared in 100 mL transparent serum bottles filled with the same liquid media, and sterilized. The bottle containing media was placed with a 2% inoculum in darkness for 24 hours to allow any residual oxygen to be used, thus avoiding possible oxidative damage to cells when they were placed at 30°C under 2,000 Lx white light. The procedures for anaerobic cell growth were done similarly to those under aerobic conditions. Cell cultures under anaerobic conditions were grown at 30°C for 120 hours with 2,000 Lx white light. The effect of different carbon sources on catalase activities was examined only under aerobic conditions in this study. Cells were pre-cultured aerobically on PYS medium [0.3%(w/v) peptone, 0.3%(w/v) yeast extract, 2 mM $MgSO_4$, 2 mM $CaCl_2$] to the exponential growth phase, thereafter, 1 mL of the pre-culture was inoculated onto each of 0.3% acetate-, ethanol-, fructose-, glutamate-, lactate-, pyruvate-, and succinate-containing basal medium (3), and then incubated as described above. In order to achieve similar cell growth phase, cells were incubated for 72 hours for fructose, glutamate, pyruvate, and succinate, and for 120 hours for acetate, ethanol, and lactate due to different growth rates.

Preparation of crude extracts

Cells were collected by centrifugation, washed with 50 mM potassium phosphate buffer (pH 6.8) containing 1 mM EDTA, and resuspended in the same buffer. The resuspension was broken by the sonicator (Bandelin Sonoplus HD 2070, Germany). Sonicated cells were centrifuged at 10,000 × *g* for 30 min at 4°C. The supernatant was used as crude extracts for the enzyme assay.

Fractionation of bacterial cells

Two milliliters of the cultured cells were collected by centrifugation, and used for fractionation into periplasmic and cytoplasmic proteins according to the method of Ames *et al.* (1). To isolate periplasmic proteins, 20 μL of chloroform was added to the pellets, vortexed briefly, and incubated at room temperature for 15 min. Two hundred μL of 0.01M Tris-HCl (pH 8.0) was then added, centrifuged at 6,000 × *g* for 20 min at 4°C, and then the supernatant was withdrawn with a Pasteur pipette for analysis of periplasmic proteins by native PAGE analysis. The remaining cell pellets were suspended in 50 mM potassium phosphate buffer (pH 6.8) containing 1 mM EDTA, sonicated, and then the supernatant was used for analysis of cytoplasmic proteins.

Evaluation of catalase activity

Catalase activity was measured spectrophotometrically according to the method of Beers and Sizer (2). The activity was evaluated by monitoring the amount of H_2O_2 in A_{240} resulting from the elimination of H_2O_2, and the amount of catalase activity that decomposed 1μmole of H_2O_2 per min was defined as 1 U of activity. The extinction coefficient (ε) for H_2O_2 in 240 nm was 43.6 M^{-1} cm^{-1}. The amount of protein was determined by the method of Lowry et al (35) using bovine serum albumin as the standard size marker.

Polyacrylamide gel electrophoresis (PAGE) Analysis

Non-denaturing PAGEs of crude extracts from strain S1 were performed on 5-10% polyacrylamide gels in Mini-Protein II dual slab cells (Bio-Rad, Hercules, CA.) according to the method of Laemmli (28), or Shi and Jackowski (46) with minor modifications. Catalase activity on the gel was visualized by staining using ferricyanide as previously described (54), and peroxidase activity was visualized by the method of Claiborne and Fridovich (6) using o-dianisidine.

Determination of molecular weights of native catalases

The molecular weights of native catalases were determined basically by the method of Hedrick and Smith (18). Crude extracts of cells were electrophoresed on a series of acrylamide gels ranging from 5 to 10% concentrations. The R_f of each protein relative to bromphenol blue tracking dye was determined in each acrylamide gel of different concentrations. The slope for each protein was determined from the graph of 100× log (R_f× 100) against gel concentration. The slope was then plotted as a function of the molecular weight of the protein. The gels were stained with Coomassie blue for determination of molecular mass markers, and for catalase activity as mentioned above. The protein size markers used were the Sigma molecular weight marker kit for non-denaturing polyacrylamide gel electrophoresis: urease hexamer, 545 kDa; trimer, 272 kDa; bovine serum albumin dimer, 132 kDa; monomer, 66 kDa; chicken egg albumin, 45 kDa, bovine erythrocyte carbonic anhydrase, 29 kDa ; and bovine milk α-lactalbumin, 14.2 kDa.

Other methods and chemicals

The substrate affinity (Km value) of catalase in crude extract was determined with different concentrations of H_2O_2 of 1 mM to 60 mM. Effects of H_2O_2 concentration, temperature, and pH on the catalase activity were assayed routinely (26, 29, 37, 50). Four kinds of buffers, e.g., 50 mM citrate-phosphate buffer (pH 3.0-6.0), 50 mM potassium phosphate buffer (pH 6.0-7.0), 50 mM Tris-HCl buffer (pH 7.0-9.0), and 50 mM carbonate buffer (pH 9.0-11.0) were used (50) to evaluate the effect of pH on catalatic activity. Survival of *R. rubrum* S1 cells and the change in catalase activities in response to extracellular

hydrogen peroxide were also determined as described above. Most chemicals were purchased from Sigma Chemical Co., (St. Louis, MO).

Results and Discussion

Identification of catalases and peroxidases in R. rubrum S1

Catalase activity staining for proteins from *R. rubrum* S1 grown aerobically in the dark revealed five distinct bands (Fig. 1A), which were designated Cat 1, Cat2, Cat3, Cat4, and Cat5 in the order of higher molecular weight. However, cells grown anaerobically in the light exhibited only three distinct bands, Cat 2, Cat 3, and Cat 4 (Fig. 1B). Peroxidase activity staining for the first five bands revealed that Cat 3 and Cat 4 have peroxidase activities, too. From this result, we concluded that *R. rubrum* S1 could produce three monofunctinal typical catalases and two bifunctional catalase-peroxidases. Various types of catalases from bacteria have been found in several species. Most bacterial species were found to produce three or fewer catalases (12, 21, 23, 29, 30, 32, 34, 56, 57), while *Streptomyces coelicolor* was found to have six catalases (26), and *Pseudomonas syringae*, eight catalases (27). It is notable that the photosynthetic bacteria produce one or two catalases. As an example, *Rhodobacter sphaeroides* and *Rhodobacter capsulatus* produce only one catalase (7, 10, 37, 50). Nadler *et al.* (37) reported that *R. rubrum* S1 produced only one typical catalase under anaerobic conditions. It is assumed that the typical catalase might be identical to Cat2 identified in this study. This result is strikingly different in the number of catalases from that obtained in our study. The discrepancy might result from the possibility that they could not recognize other catalases (Cat 3, 4) that have much lower activity than Cat2 activity. Above all, the great difference is that our data were obtained through analyses of catalases from the cells grown under both aerobic and anaerobic conditions, while they used only anaerobically grown cells. The number of catalases in even a particular bacterial species can be changed by various internal or external factors such as growth phase (4, 26, 33, 36, 42, 47), oxygen (10, 20, 22, 36, 42, 43), H_2O_2 (20, 30, 42), pH (56), carbon source (42, 43, 47), starvation (42) or complex factors (21, 36, 42). The presence or absence of oxygen is the principal difference between aerobic and anaerobic cultures. Accordingly, it could be postulated that the difference in the number of catalases between aerobically and anaerobically grown S1 cells might be caused by oxidative stress.

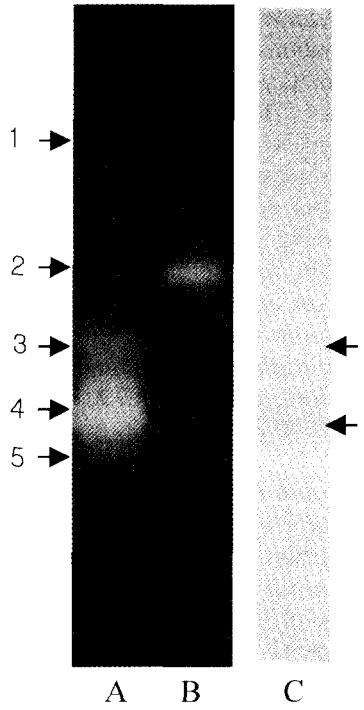


Fig. 1. Identification of catalases and peroxidases by nondenaturing PAGE (7% acrylamide) of the crude extracts from *R. rubrum* S1. Catalases were assayed from S1 cells grown under both aerobic (A) and anaerobic (B) conditions. Peroxidase activities were assayed with crude extracts of S1 cells only under aerobic conditions (C).

On the other hand, anaerobically grown S1 cells produced a fewer number of catalases and lower catalase activity than aerobically grown cells. However, it is remarkable that S1 cells exhibited much stronger Cat 2 activity in response to the anaerobic condition than to the aerobic condition. Some previous reports on catalases production by anaerobically grown bacteria including photosynthetic bacteria (10, 22, 37, 42, 43, 50) raised questions about the major function of catalases in the anaerobic environment. It has been reported that external oxidative stresses, such as H_2O_2, oxygen, or benzyl violet enhanced catalase production in *R. sphaeroides* (50) and *Bacteroides fragilis* (42). However, the role of intrinsic catalase in the photosynthetic bacteria, or obligate anaerobic bacteria that live in anaerobic environments without any external oxidative (or -related) stresses is still obscure (7, 37, 50). Rocha and Smith (42) suggested that starvation might be a catalase inducer in anaerobically grown *B. fragilis* in stationary-phase anaerobic bacterium, and that an unknown metabolite also could be a catalase inducer, because the stationary growth phase has two facets, nutrition and metabolite. Considering combinations of these previous data, the stronger expression of Cat2 in *R. rubrum* S1 grown in the anaerobic environment might result from several factors such as an unknown metabolite including any kind of free radical, starvation, light, or property of minimum provision against unexpected exposure to oxidative stress.

Molecular weights of catalases

The molecular weights of Cat2, Cat3, Cat4, and Cat5 in strain S1 through non-denatured PAGE analysis were estimated at approximately 323 kDa, 266 kDa, 246 kDa, and

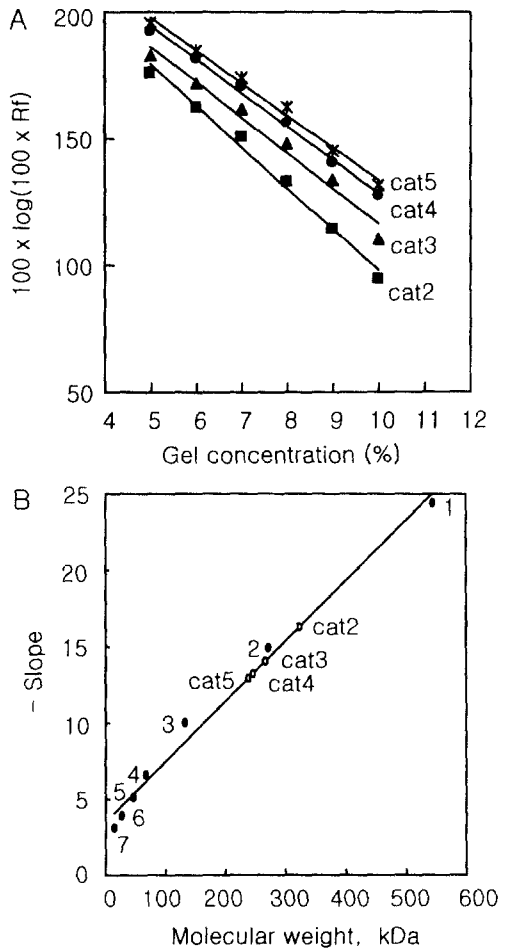


Fig. 2. Determination of molecular weights of catalases of *R. rubrum* S1 by nondenaturing PAGE. Mobility of catalases, Cat2 (■), Cat3 (▲), Cat4 (●) and Cat5 (*) in different concentrations of acrylamide gel (A). Determination of the molecular weight of the four catalases based on the slopes determined in panel A. The protein marker; 1, urease (hexamer, 545 kDa); 2, urease (trimer, 272 kDa); 3, bovine serum albumin (dimmer, 132 kDa); 4, bovine serum albumin (monomer, 66 kDa); 5, chicken egg albumin (45 kDa); 6, carbonic anhydrase (29 kDa); 7, α-lactalbumin (14.2 kDa) (B).

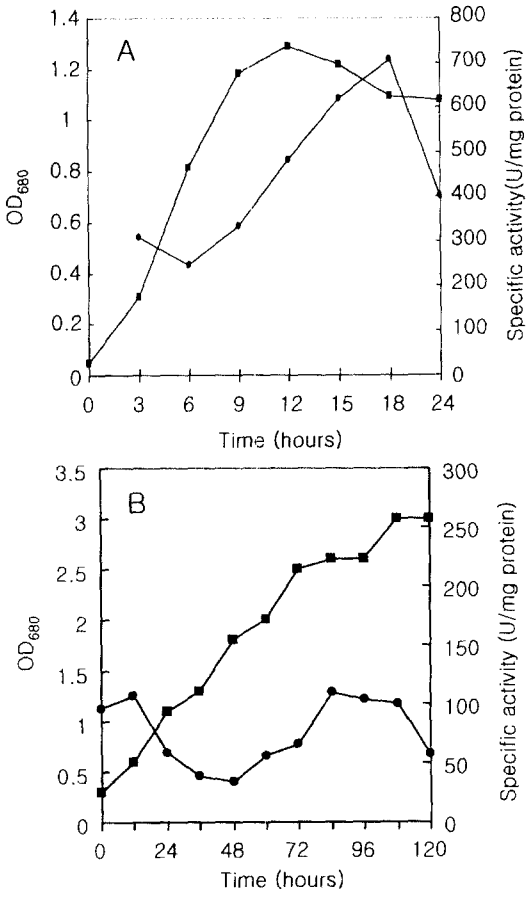


Fig. 3. Cell growth (■) and catalase activity (●) of crude extracts from *R. rubrum* S1 cultured under aerobic conditions (A) and anaerobic conditions (B). Data are the means of two separate experiments.

238 kDa, respectively (Fig. 2 B). The MW of Cat 1 was assumed to be over 350-400 kDa, based on the relative distances of catalase bands on the gel (Fig. 1A) even though it could not be determined exactly because of the unusual electrophoretic mobility behavior. The MWs of catalases from a variety of bacteria including photosynthetic bacteria are mostly in the range of about 200 kDa to 300 kDa with some exceptions (4-7, 10, 12, 13, 21, 26, 29, 34, 40, 42, 47, 50).

Growth phase and catalases activities

R. rubrum S1 cells, when grown under aerobic conditions, exhibited the lowest catalase activity (247 U/mg protein) at the mid-logarithmic growth phase, and the highest activity (706 U/mg protein) at the mid-stationary growth phase. The catalase activity rapidly decreased immediately after the mid-stationary phase (Fig. 3A). S1 cells grown under anaerobic conditions exhibited the lowest activity (34 U/mg protein) at the early-logarithmic growth phase, while the highest activity (110 U/mg protein) occurred at the early-stationary growth phase. Total catalase activity rapidly decreased at the early stationary phase (Fig. 3B). Comparative analyses of enzyme activities, based on the lowest and highest catalase activities, revealed that S1 cells grown under aerobic conditions exhibited 6.8- and 6.6-fold higher activities than those found in anaerobically grown cells, respectively. These results obtained from crude extracts of both aerobic and anaerobic cultures were consistent with the band intensities obtained visually through non-denatured PAGE (Fig. 1A, B). Many bacterial species, such as *S. coelicolor*, *Bacillus subtilis*, *B. fragilis*, and *Pseudomonas aeruginosa*, showed similar patterns of catalse activity in each phase of cell growth. Many studies reported that cells at the late-logarithmic phase or stationary phase produced much greater numbers of catalase, as well as higher catalase activities than cells at the lag phase or early-to mid-logarithmic phase (26, 27, 32, 34, 42, 43, 49). In contrast to

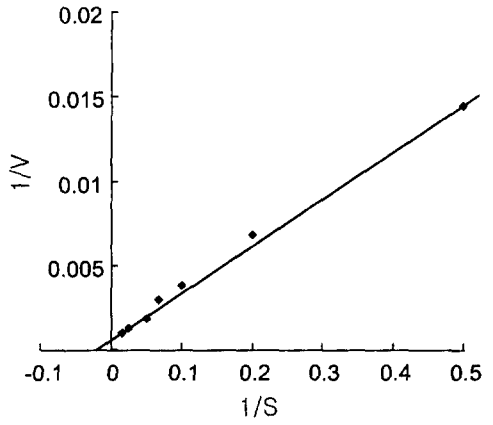


Fig. 4. Lineweaver-Burk plot of the reaction velocities of the cataleses from *R. rubrum* S1. The enzyme assay was performed at various concentrations of hydrogen peroxide under standard assay conditions as described in Materials and Methods.

other bacteria, there were no changes in the number or type of catalases over the whole cell growth phase of *R. rubrum* S1, even though there were fluctuations in catalase activity (data not shown). A Lineweaver-Burk plot of catalase in crude extracts from aerobically grown S1 cells showed about 10 mM of Km value for H_2O_2 (Fig. 4). Generally, typical catalses have high Km values of about 40-80 mM (23, 33, 50), while atypical catalase-peroxidases have low Km values of 1-10 mM for H_2O_2 (6, 27, 56). The lower Km value of crude extracts from *R. rubrum* S1 for H_2O_2 suggested a possibility that the catalase activity of aerobically grown S1 cells might be controlled mainly by Cat 3 and Cat 4 that are bifunctional as catalase and peroxidase.

Effect of pH and temperature on catalase activity

Catalase activity was assayed at various pHs and temperatures using crude extracts of aerobically grown S1 cells (Fig. 5A and Fig. 5B). The optimum temperature for maximum catalase activity in strain S1 was approximately 30°C, and about 50% of the maximum activity was observed at 60°C. Incubation of crude extracts from aerobically grown cells at 70°C resulted in the rapid suppression of catalase activity. Catalase activity of crude extract from aerobically grown S1 cells was found to be stable in the broad pH range from pH 6.0 to 9.0. It has been reported that typical catalases were stable in the broad pH range from pH 5.5 to 10.0, while an optimum pH range for atypical catalase-peroxidase activity was at ≅ pH 6 (12, 37, 50). Catalases or catalase-peroxidases of photosynthetic bacteria, e.g., *R. rubrum*, *R. sphaeroides*, *R. capsulatus*, and *Chromatium vinosum*, were found to exhibit activity at a similar pH range as in other bacteria (24, 37, 50). Atypical catalase-peroxidases from *R. capsulatus* and *Escerichia coli* were found to be more sensitive to temperature than typical catalase (37). These

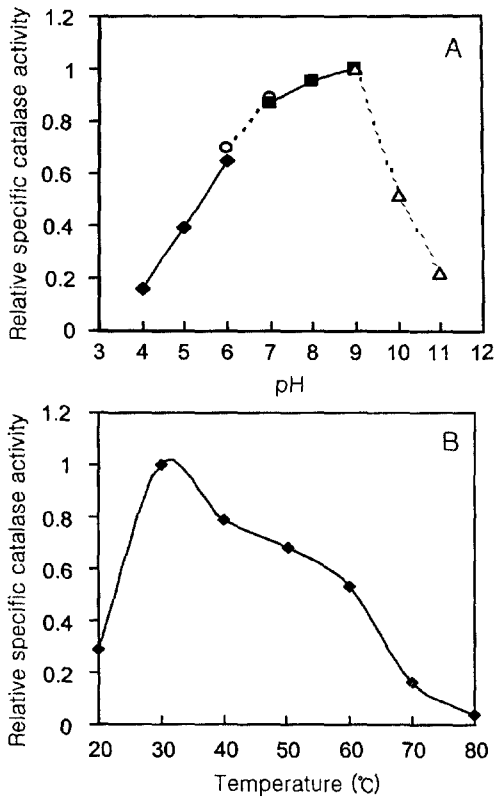


Fig. 5. Effect of pH and temperature on the catalase activity of crude extracts *from R. rubrum* S1. (A) crude extracts were incubated for 10 min at 30°C prior to the initiation of the reaction. Different buffer systems were used according to pH ranges; 50 mM citrate-phosphate buffer (◆) for pH 3.0-6.0, 50 mM potassium phosphate buffer (○) for pH 6.0-7.0, 50 mM tris-HCl buffer (■) for pH 7.0-9.0, and 50 mM carbonate buffer (△) for pH 9.0-10.0. (B) Crude extracts were incubated for 10 min at the indicated temperatures prior to the initiation of the reaction.

results suggest that S1 catalases shared many similar properties with those found in other bacteria.

Effect of H_2O_2 on the catalase activity and survivals

The relative specific catalase activities from crude extract of aerobically grown S1 cells at the exponential or stationary phase with different concentrations of H_2O_2 was shown (Fig. 6A). The catalase activities of cells at the exponential phase were significantly decreased by treatment with 10 to 20 mM H_2O_2 for 1 hr immediately before the enzyme assay. In contrast, when cells at the stationary phase were treated with 5 to 20 mM H_2O_2, the catalase activity remarkably increased, but decreased in response to higher concentrations over 20 mM of H_2O_2 (data not shown). The results indicated that cells exposed to H_2O_2 at the stationary growth phase produce much more catalases than those of at the exponential growth phase. Similar results were obtained in many studies on catalases from *E. coli*, *P. aeruginosa*, *R. sphaeroides*, *Lactobacillus sakei*, *P. syringae*, and *S. coelicolor*, etc. (4, 20, 27, 30, 32,

50). To study the susceptibility of *R. rubrum* S1 to exogenous H_2O_2, % survivals of S1 cells in the early exponential or stationary growth phase were evaluated by H_2O_2 treatment (Fig. 6B). Cells at both early exponential and stationary phases showed similar survival rates of about 50% against 5 mM H_2O_2. However, survival rates of cells at the exponential phase significantly decreased to 3.6% at 10 mM H_2O_2, while cells at the stationary phase exhibited 25% survival rates. Even at 120 mM H_2O_2, cells at the stationary phase exhibited approximately 10% survival rates, but survival rates at the exponential phase were below 0.1%. These results suggest that cells at the early exponential phase were more susceptible to H_2O_2 than those at the stationary phase. Data were consistent with those found for *R. capsulatus*, phytopathogenic strains of *Pseudomonas syringae*, *S. coelicolor* and *Caulobacter crescentus* (22, 27, 30, 44). As a result, this study provided additional evidence that there is a relation between catalase activity and cell growth phase, and that catalase exhibits maximum activity in the stationary growth phase of S1 cells.

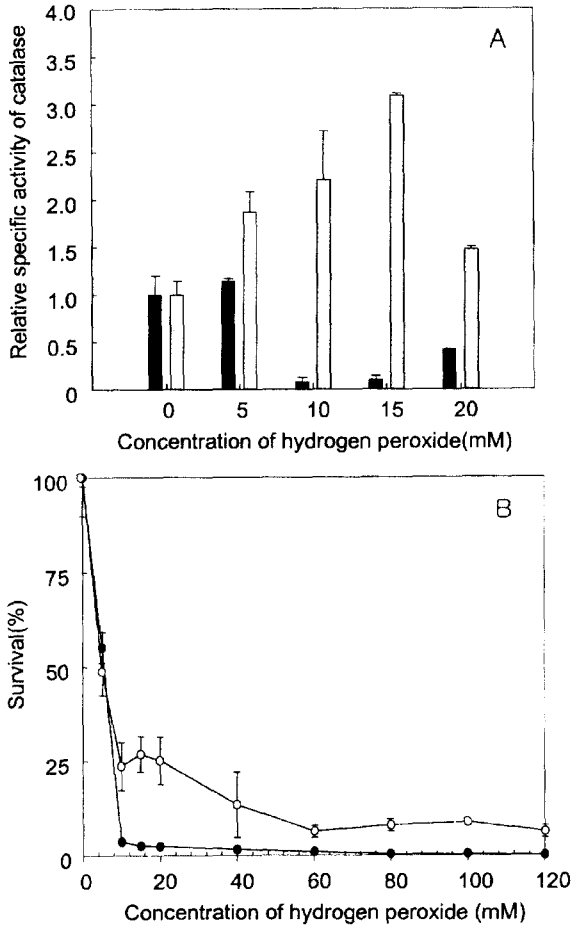


Fig. 6. Effect of hydrogen peroxide on catalase activities and survials of S1 cells grown under the aerobic conditions. (A) Effect of hydrogen peroxide on the catalase activities of crude extracts from aerobically grown *R. rubrum* S1 cells of exponential (■) and stationary phases (□). Cells at different growth phases were treated with hydrogen peroxide of indicated concentrations for 1hr before harvest and activity assay. The values are relative ones for untreated controls (defined as 1.0). (B) Percent survials of *R. rubrum* S1 cells in the early exponential (●) and stationary growth phase (○) after 1 h exposure to hydrogen peroxide of various concentrations. Data are the means of three experiments (± SE).

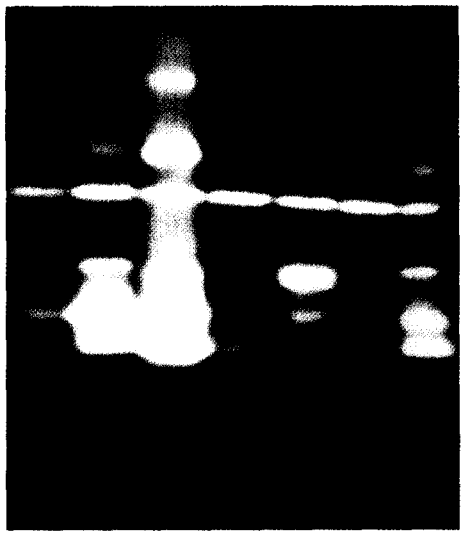
Effect of carbon sources on catalase synthesis

The effects of various carbon sources on the synthesis of catalases in *R. rubrum* S1 were examined (Table 1 and Fig. 7). Addition of 0.3% L-glutamic acid to the growth medium resulted in an 80-fold increase of catalase activity over 0.3% malic acid. As seen in Fig. 7, Cat 2 that was much more strongly expressed under anaerobic conditions

Table 1. Effect of various carbon sources on catalase activity of *R. rubrum* S1[1]

Carbon source	Catalase specific activity (U/mg protein)	Cell yield[2] (OD_{680})
Malic acid	25.47	1.328
Fructose	35.66	0.974
Lactate	595.01	0.827
L-Glutamate	2097.85	1.071
Succinate	65.48	0.983
Pyruvate	199.77	1.032
Ethanol	27.23	0.543
Acetate	251.72	0.302

[1]Catalase activities were assayed with S1 cells grown under aerobic conditions.
[2]Values of cell yield were determined with the maximum cell density at the stationary phase.



A B C D E F G

Fig. 7. Activity staining of catalases after non-denaturing PAGE of crude extracts from *R. rubrum* S1 grown on various carbon sources. Lane (A) fructose; (B) lactate; (C) L-glutamate; (D) succinate; (E) pyruvate; (F) ethanol; (G) acetate. *R. rubrum* S1 precultured on PYS medium was inoculated into the new media containing each of the different carbon sources indicated above for the main culture.

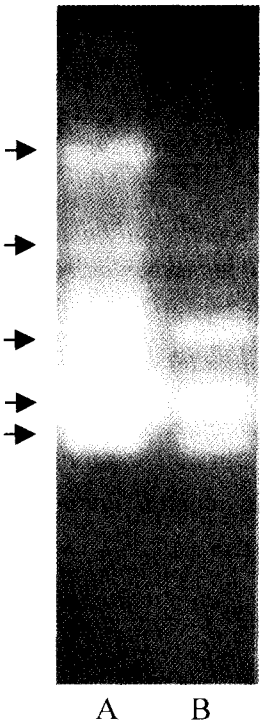


Fig. 8. Activity staining of catalase after non-denaturing PAGE (7%) of cytoplasmic (A) and periplasmic (B) fractions from aerobically grown *R. rubrum* S1 cells.

was constantly synthesized regardless of carbon sources. However, expression of other catalases fluctuated depending on carbon sources. This finding suggests that Cat2 might play a key role as a house-keeping protein in various environments. Several microbiologists have reported that the carbon source could affect the synthesis of catalase in *E. coli* (17), *Acinetobacter* sp. (47), and even the anaerobic bacterium, *B. fragilis* (43). Our results obtained from *R. rubrum* S1 provided additional information that a carbon source might be a potential regulatory factor for catalase gene expression in bacteria.

Localization of catalases in the cell

Analysis of non-denaturing PAGE of cell fractions from aerobically grown S1 cells at the stationary phase revealed distinct catalase bands from the periplasm and cytoplasm (Fig. 8). All kinds of catalases mentioned above, Cat1, Cat2, Cat3, Cat4, and Cat 5 were observed in the cytoplasmic fraction, while only Cat1 was excluded in the periplasmic fraction. In previous studies on the localization of catalases, atypical catalase-peroxidase, HP I, was found in both periplasmic and cytoplasmic fractions of *E. coli*, but typical catalase, HP, was observed only in the cytoplasmic fraction (19). *P. syringae* cell contained six catalases in the cytoplasmic and four catalases in the periplasmic fraction, and two of them were found in both fractions (27). Similar studies of *C. crescentus*, *Vibrio rumoiensis*, and yeast *Schizosaccharomyces pombe* (31, 44, 57) also revealed catalases found in both cytoplasm and periplasm. The difference between our result and the previous data is that most of the catalases from S1 strain were in both the cytoplasm and periplasm, suggesting the possibility that *R. rubrum* capable of aerobic and anaerobic growth might have a much quicker and more effective defense system against possible external stresses.

Acknowledgments

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